

Dear Maxine -

Dec 28

What a lovely Christmas present! I was certainly surprised and delighted to find a copy of the "Sandburg Range" under our tree — such unexpected fun! Bill Bowen has a copy of a book called, I believe, the "Lincoln Reader" which also consists of selected writings of Carl Sandburg and we read a few excerpts. They were quite enjoyable. You didn't indicate where the time for reading the book is to be found. It's deceptive to state that it can be done in small portions for the same total amount of time is involved. It's true, as you suggest, that some of it could be read to David during time allotted for the children, but he wouldn't stand for the poetry sections.

I hope that you are having a wonderful time in the islands. In a way it's too bad that your trip didn't happen to have occurred in January or February when the weather is Washington is bitter cold. Actually, we've had some balmy, sunny days and some more beautiful sunsets. February will be another story. That's the time to get away.

The boys were wonderfully excited Christmas morning and quite pleased by their presents. David got two "Hardy Boys" books, an HO train set, a fishing rod, several plastic models, an Ivy League shirt (of which he's fond), an aeroplane which rotates on a rod under some mysterious electronic control. Alan got a Roy Rogers chuck wagon set, a Roy Rogers stagecoach set, a "Sad Dog" piggy bank, a plastic tea set, a set of cooking utensils, fishing rod, a book on "Bambi". David also got a record of "Around the World in 80 Days". This rather respectable travel results from having three uncles and four aunts and also from the fact that both boys also have birthdays in December.

I've been working terrific hours this past week and cut my

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sleeping to seven hours. I find I bear up quite well as long as I'm rude to visitors and don't answer the telephone. Interruptions, not work itself, exhausts a person.

On the whole the experiments have turned out quite well. The results are as follows:

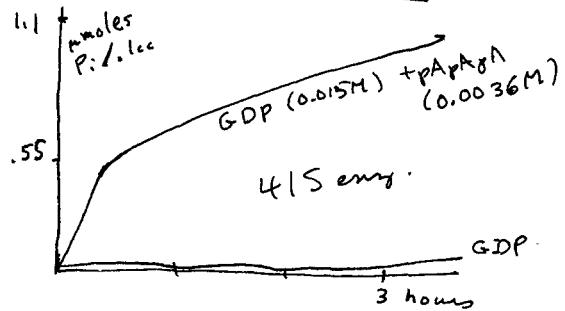
The mononucleotides resulting from alkaline digestion of poly nucleotide made with GDP + pApA or GDP + pApApA are surely the mixed 2' and 3' isomers. They are split by serum esterase but not by bull semen 5'-nucleotidase and they have the right R_F in C₈₀A and I.P.NH₃. Guanosine derived from one "end group" can just barely be detected, with the Mineralite lamp its bluish rather than black and blends in with the background. pAp for the "front end" of pApApGpGpT hasn't been looked for as yet. A complication is that GDP and GMP are trapped with "origin material" and I'm not sure that pAp resolves when mixed with there. I've thought of incubating GDP + pApA + poly nucleotide phosphorylase and then adding GDP and 5'-nucleotidase, forming guanosine from residual GDP and GMP. It should be practical.

These guanosine poly nucleotides are interesting. They don't precipitate out of the incubation mixture, yet their solubility in water is very limited. Elution off paper is difficult and they precipitate in the tube containing eluting fluid. Apparently salts contained in the chromatograms help dissolve the material of $R_F = 0$ and subsequent dilution by the eluting water gives precipitation. Some of the material never does elute even with hot water or NH₃. By chilling the elution fluid one obtains a beautiful ~~the~~ crystalline precipitate. Markham has observed this for G containing dinucleotides. Maybe PO₄²⁻ or GDP helps keep the material in solution.

With pApApA and GDP one gets material of $R_F = 0$ and two other bands of low R_F . The slower moving gives mostly AMP, 2' & 3' with alkali and just a little GMP, 2' & 3'. The faster moving band gives AMP, 2' & 3', no detectable GMP, 2' & 3', and faint densities for guanosine and pAp. Presumably it's pApApApG.

Some results:

12/27



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$GDP = 1.5 \mu\text{moles} / 0.1 \text{cc}$, so reaction proceeds up to 70% completion. Quantitative serial chromatograms in protos. No visible changes for GDP alone. For $GDP + pApApA$ ($3.6 \mu\text{moles } pApApA / 0.1 \text{cc}$)

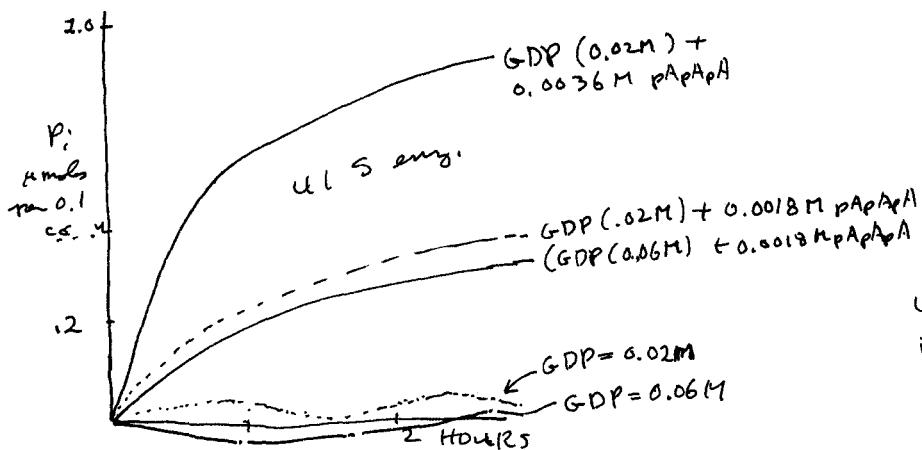
40 MIN No origin material in $IPNH_3$ or Krebs-Hems Faint density in Krebs-Hems above GDP .

Heavy density just below 5'GMP in Krebs-Hems — it must be $pApApApG$. $pApApA$ about 2/3 incorporated.

140 MIN similar — 3/4 incorp.

210 MIN nearly all incorp. intense origin density — still a lot of (?) $pApApApG$

12/26



This run shows that no saturation for $pApApA$ has been achieved, most likely. Saturation is at least $0.0036M$. With latest batch of GDP there was no reaction without primer, even at $0.06M$. With an older lot, there was indication of a rise in P_i after a lag.

12-26, 12-27, 12-25 — repeated runs were made with purified venom on "origin" material and (?) $pApApApG$. Products are 5'-GMP and 5'-AMP — further evidence on their identity. 5'-GMP appears to be released first, but it's hard to get the enzyme level just right.

12/24 $GDP = 0.025M$, with and without $pApA$ ($0.21 \mu\text{moles } pApA \text{ per } 0.1 \text{cc}$) E. coli eng.

	GDP	$GDP + pApA$
55'	0.15	0.21
7 hours	0.15	0.53

26½ hours 0.28 1.09

Chromatogram shows perhaps $1/3 - 1/2$ of $pApA$ still present after some hours. At least 5 units of G are added /molecule primer — maybe more

12/23 Mon Enzyme fraction 41S. 7 tubes, 35', 2 hours, 4 hours. $GDP = 0.015M$. Results show stimulation by $0.0013M$ $pApApA$. With $0.0004M$ pantetheotide got no stimul. at 35', 2 hours and at 4 hours got a little rise ($125 \mu\text{moles } P_i / 0.1 \text{cc}$).

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With ApApUp (0.0008M as tri) got absolutely nothing. With Michelsons polyA got no effect. With pApApA and enzyme varying 0.003 $\frac{1}{10}$, 0.005 $\frac{1}{10}$ and 0.015 $\frac{1}{10}$ got fair enzyme-rate proportionality.

12-21 - Sat Tested adenosine for Herbr and made "polymer" with pApA and pApApA for alkali treatment.

12-20 — FRI You helped set this up but left before seeing results. sa 150 Enzyme, Poly A + ADP same as usual. ApApUp at 0.0008 M (always as compound itself) got stimulation not quite as good as polyA at 0.1mg per 0.1cc inc. mix. but still very good. Michelsons poly at 0.15 mg / 0.1cc inc. mix. was about same as ApApUp. Russell's RNA "rose" at 0.15 mg / 0.1cc was almost negative. ApApUp at 0.0002 M was perhaps 2/3 as good as at 0.0008 M.

Now that I've gotten used to the idea that ApApUp, which apparently isn't incorporated, really stimulates the reaction it's beginning to fascinate me. As usual, no incubation mix. was obviously viscous except the two with ApApUp and they were really viscous. How can a compound like this act to stimulate the reaction?

We're spending Mon. & Tues in Ephrata. The boys are quite excited about the visits. David looks very sharp in a new tux jacket shirt, of which he's very proud.

The Ann. Rev. article still wasn't finished when I left. In 3½ days Rose managed to type exactly 18 pages — an awful record. I got disgusted and typed up the Fed. Abstract myself. Following your suggestion, it mentions nothing about stimulation — only incorporation data & venom results.

Thanks again for the lovely book. I plan to delve into it while up here in Penna. & read out of it to the boys

Best to Denny

Sincerely, John